Food Chemistry: X 20 (2023) 100932

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Comparative characterization and contribution of key aroma compounds in the typical base liquor of *Jiang*-flavor *Baijiu* from different distributions in the Chinese Chishui River basin

Jiaxin Gong [a,b], Yu Ma [a], Lili Li [a], Yuxin Cheng [a,b,*], Yongguang Huang [a,b,*]

[a] College of Liquor and Food Engineering, Key Laboratory of Fermentation Engineering and Biological Pharmacy of Guizhou Province, Guizhou University, Guiyang, Guizhou 550025, China

[b] Key Laboratory of Fermentation Engineering and Biological Pharmacy of Guizhou Province, Guiyang, Guizhou 550025, China

ARTICLE INFO

Keywords:
Jiang-flavor *Baijiu*
Producing core region
Typical base liquor
Odor activity value
Aroma recombination

ABSTRACT

The characteristic of typical base liquor is crucial in controlling ultimate quality of *Jiang*-flavor *Baijiu*. This study investigates the flavor compounds of three typical base liquors (*Jiangxiang*, *Chuntian*, and *Jiaodixiang*) by LLE/LLME/HS-SPME, gas chromatography–mass spectrometry (GC-MS), gas chromatography–flame ionization detection (GC-FID), sensory analysis, and odor activity value (OAV). Of the 201 main volatile compounds identified, 37 significant compounds distinguished the three typical base liquors. Acid (441.72 ± 0.17 mg/L), alcohol (5388.88 ± 0.55 mg/L), and ester compounds (8181.64 ± 0.15 mg/L) were respectively marked in *Jiangxiang*, *Chuntian*, and *Jiaodixiang* typical base liquors. Orthogonal partial least squares discriminant analysis (OPLS-DA), correlation analysis, and aroma recombination showed that butyric acid (OAV: 102.23), butyl 2-methylbutyrate (OAV: 6045.59), and ethyl caproate (OAV: 418.37) were significantly correlated with sweet, fruity, pit mud, *jiang*, and ethanol aromas. It identifies the primary constituents that affect flavor variations in the three typical base liquors and provides guidance for investigations on the flavor formation of *Jiang*-flavor *Baijiu*.

1. Introduction

Alcoholic beverages (such as beer, wine, and spirits), owing to their rich flavor compounds, are distinctive foodstuffs in human life around the world (Precieuse, Kumar, Suri, Gat, & Kumar, 2018). Among them, flavor compounds are key determinants of consumer preference and a criterion for orienting the sensory characteristics of alcoholic beverages. The small molecular alcohols, aldehydes, and phenols could enhance the drinking experience. Besides, the dynamic equilibrium relationship between acids and esters in *Baijiu* is necessary for the softness and harmony of flavors (Chen et al., 2023; Li, Xu, Yu, & Zheng, 2023).

However, it is difficult to control all flavor compounds due to their large variety and content. Thus, the key aroma compounds are always selected to determine the primary flavor characteristics of alcoholic beverages according to their concentration and olfactory threshold (Luo, Kong, Xue, Wang, & Xia, 2020). In particular, the key aroma compounds in alcoholic beverages were identified as having an effect on the main dominant flavor through sensory analysis and quantitative detection (Yang et al., 2023). This suggests that the analysis of flavor substances and key active aroma compounds of flavor substances in alcoholic beverages is necessary for a better understanding of the aroma presentation mechanism.

Baijiu, the world's highest-selling distilled spirit, has various flavors (such as *Jiang*-flavor, strong-flavor, and light-flavor *Baijiu*) owing to the different fermentation processes (Zhang et al., 2022). Among them, *Jiang*-flavor *Baijiu* has a complex brewing process and a rich flavor profile with a wide variety of flavor compounds (Fig. 1). The reasons for the diversity of flavors in *Jiang*-flavor *Baijiu* are the large number of core production regions as well as the variability in the geographical location of the core production regions (Pan, Qiu, Lv, & Li, 2023). Many famous *Jiang*-flavor *Baijiu* liquor companies are located in the Chishui River basin in Chinese Guizhou, which is the core production area of *Jiang*-flavor *Baijiu* because of the unique geographical environment. The similarities and differences of *Jiang*-flavor *Baijiu*' flavor compounds in various regions will be understood by analyzing the typical base liquor of the upstream, midstream, and downstream regions (*Xishui*, *Jinsha*,

* Corresponding authors at: College of Liquor and Food Engineering, Key Laboratory of Fermentation Engineering and Biological Pharmacy of Guizhou Province, Guizhou University, Guiyang, Guizhou Province 550025, China.
E-mail addresses: yxcheng3@gzu.edu.cn (Y. Cheng), yghuang1@gzu.edu.cn (Y. Huang).

https://doi.org/10.1016/j.fochx.2023.100932
Received 16 July 2023; Received in revised form 18 September 2023; Accepted 6 October 2023
Available online 11 October 2023

Maotai-bianyuan, *Maotai-hexin*, and *Donggongshi*) of the Chishui River basin. The fermentation environment and microbial structure of *Jiang*-flavor *Baijiu* in the pit were different (Liu et al., 2023), resulting in the distillation of *Baijiu* from various layers of fermented grains in the upper, middle, and lower layers of *Baijiu* being divided into three different typical base liquors (*Jiangxiang*, *Chuntian*, and *Jiaodixiang*). The study of flavor compound structures and flavor characteristics in these three typical base liquor can help us better understand the scent and flavor mechanisms of *Jiang*-flavor *Baijiu* styles and their flavor compounds (Xu et al., 2023).

The role of the typical base liquor has been clarified previously (Xiong, Li, & Ma, 2022). Nevertheless, research on the compounds of a typical base liquor was restricted to quantitative analysis (Li, Ma, Huang, You, Cheng, & Hu, 2021; Liu, Zhao, Li, Zhang, Zhang, & Liu, 2018), and only a few different kinds and amounts of volatile compounds were found in the three typical base liquors. Volatile compounds obtain different characteristics in terms of polarity, solubility, and volatility. Thus, there is no single pretreatment approach that could totally extract all volatile compounds from *Baijiu*. A multitude of pretreatment methods is commonly utilized to treat the sample to largely reflect and characterize the volatile compounds and overall aromatic profile of the sample (Coelho, Lemos, Genisheva, Domingues, Vilanova, & Oliveira, 2020; Ma, Gao, Chen, & Meng, 2020). Additionally, the sensory analysis combined with the aroma recombination test is essential for revealing the actual aroma contribution of key compounds (Liu, Yang, Wang, & Song, 2021). Although rounds 1–7 of different quality levels forming the three typical base liquors have been revealed (Wu et al., 2023). While the flavor profiles and flavor compounds of the three typical base liquors originating from the main production area of *Jiang*-flavor *Baijiu* have not been clearly scientifically demonstrated.

Therefore, this study aimed to (1) comprehensively investigate the key flavor compounds in the typical base liquor from the Chishui River basin by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID); (2) understand how the key flavor compounds contribute to *Baijiu* flavor and how they affect sensory perception by combining the sensory analysis with a recombination test; and (3) reveal the source path of key compounds through the correlation between the content of compounds and flavor. Overall, this study provides insights into the different uses of different typical base liquor in the blending and quality control of the *Jiang*-flavor *Baijiu*.

2. Materials and methods

2.1. Sample collection

A total of 378 samples (1000 mL of each) were collected in 2021–2022 from 1–7 rounds of three typical base liquor from 21 companies in five different core production regions of the Chishui River basin (including the *Jinsha* region (JS), the *Maotai-hexin* region (MH), the *Maotai-bianyuan* region (MB), the *Xishui* region (XS) and the *Donggongshi* region (DGS)). Each sample of a typical base liquor (140 mL) was a mixture of 1–7 rounds of base *Baijiu* (20 mL of each). Finally, the *Jiangxiang* (56 ± 0.4% v/v), *Chuntian* (56 ± 0.2% v/v), and *Jiaodixiang* typical base liquor (56 ± 0.5% v/v) were obtained. All the samples were stored at 4 °C.

2.2. Reagents and chemicals

The compounds (99.99%), including 2-octanol, *n*-amyl acetate,3-methylbutanoic acid ethyl ester, propyl hexanoate, etc., were purchased from Aladdin (Shanghai, China). Sodium chloride, anhydrous sodium sulfate, concentrated hydrochloric acid, and sodium bicarbonate (analytically pure) were purchased from Sinopharm Shanghai Co., Ltd. Anhydrous ethanol, *N*-alkane mixed standard C5-C30, and *N*-alkane mixed standard C7-C40 were purchased from Sigma-Aldrich Company (Shanghai, China).

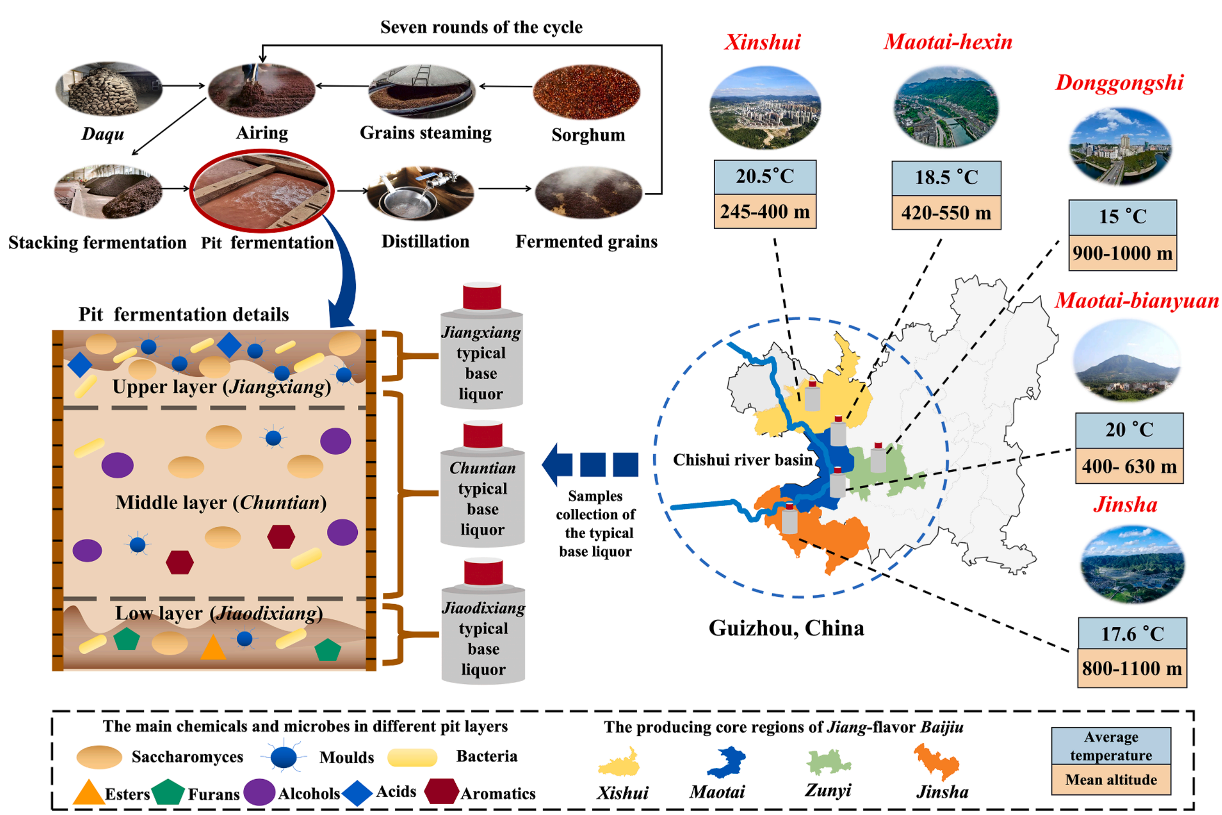

Fig. 1. Brewing process of the typical base liquor.

2.3. Sensory analysis

The sensory analysis methods were applied based on the National Standard of the People's Republic of China (GB/T 10345–2007) and previous experiments with slight modifications (He, Liu, Qian, Yu, Xu, & Chen, 2020). For *Baijiu* examination, a sensory tasting panel of 13 tastemakers was formed. All members (aged 21–45) were healthy, professionally trained, and had expertise (more than two years) in *Baijiu* taste evaluation. Two Chinese national *Baijiu* judges, six Chinese provincial *Baijiu* judges, eight grade 2 or above tastemakers, and five postgraduate students were included. The sensory module indicators, such as the sensory intensity of scents like *jiang*, caramel, and floral aroma, as well as the taste like sour, mellow, bitter, and spicy, were employed. The typical base liquor samples (20 mL each) were served in standard *Baijiu* tasting glasses and coded randomly. The sensor value (SV) was scored using a 6-point scale ranging from 1 SV (lowest intensity) to 6 SV (highest intensity) in comparison to the intensity of the references.

2.4. Volatile compounds identification from three typical base liquors

Liquid-liquid extraction (LLE) was referred to in the previous literature (Fan & Qian, 2006). Briefly, the typical base liquor sample (50 mL) was diluted to 10% (v/v) with Milli-Q water (Millipore, Bedford, MA). The diluted solutions were then saturated with NaCl. Dichloromethane was added to extract the solutions (30 mL), and the organic phases were used as component I. Component I was saturated with NaCl, followed by the addition of dichloromethane to extract the solutions (15 mL). After adding 50 mL of ultrapure water and adjusting the pH to 10, component I was divided into an aqueous phase (II) and an organic (III) phase. Component II (adjusted pH to 2) and 15 mL of ethyl ether were added to extract the acid/water-soluble component twice. Component III was extracted by adding 40 mL of ultrapure water and adjusting the pH to 2 and the aqueous phase pH to 11. Approximately, 7.5 mL of diethyl ether and pentane were added to combine the organic phase with a basic component for extraction. The basic components and acid/water-soluble components were finally concentrated to 200 μL by nitrogen blowing and pending analysis by GC-MS while maintaining the neutral fraction in the fractionation process.

Liquid-liquid microextraction (LLME) was used for the pretreatment of *Baijiu*. After the typical base liquor sample was diluted to 10% (v/v), 10 mL of each sample was taken and saturated with NaCl. The organic phase of the typical base liquor sample was extracted by shaking 2 mL diethyl ether and pentane (1:1, v/v) for 3 min. The upper organic phase was taken for GC-MS analysis after resting and separating the layer.

Headspace solid-phase microextraction (HS-SPME) was used for the pretreatment of *Baijiu* (Zhang et al., 2022). The typical base liquor sample (20 mL) was diluted to 10% (v/v), saturated with NaCl, and placed in a headspace container before incubated in a 50 °C water bath. A conditioned HS-SPME fiber was coated with DVB/CAR/PDMS (50/30 m) and subjected to the headspace for 45 min. After the extraction, the fiber was put into the GC-MS injection port and desorbed at 250 °C for 5 min.

2.5. Quantitative analysis of aroma compounds in the typical base liquor

The components obtained by the LLE/LLME/HS-SPME method were analyzed by GC-MS according to the standard curve (Supplementary Table S2). All experiments were conducted in triplicate. Each sample (1.0 μL) was analyzed after adding the internal standard. GC-MS analysis was performed on an Agilent GC8860 gas chromatograph equipped with an Agilent 5977B mass selective detector (MSD). All samples were analyzed on a DB-FFAP Column. (60 m × 250 μm × 0.25 μm; Agilent Technologies, Santa Clara, USA) Helium (99.99% purity) was used as a carrier gas at a constant flow rate of 2.0 mL/min, and the inlet temperature was 250 °C. The oven temperature was initially kept at 40 °C, held for 2 min, ramped to 100 °C at a rate of 2 °C/min, held for 2 min, finally raised to 220 °C by 5 °C/min, and held for 5 min. The temperatures of the ion source and transfer line were 230 °C and 240 °C. MS fragmentation was detected in the electron impact mode (ionization energy, 70 eV) and full-scan mode with an acquisition range of *m/z* 30–350. The solvent delay time was 7 min.

The GC-FID analysis was carried out using an Agilent GC8860 (Agilent Technologies, Santa Clara, USA) instrument coupled to an ionization flame detector (Supplementary Table S3). The sample was separated by a DB-Wax (30 m × 0.25 mm × 0.25 μm) column referred to the previous literature (Zhang, Wang, & Cheng, 2020). The injection temperature was 250 °C and the carrier gas was high-purity nitrogen. The flow rate was adjusted to 1 mL/min, the split ratio was adjusted to 37:1, and the tail blow was adjusted to 20 mL/min. The flow rate of hydrogen was 40 mL/min, the flow rate of air was 400 mL/min, and the detector temperature was 250 °C. The temperature program was maintained at 60 °C for 3 min and heated at 5 °C/min. Then the temperature was raised to 230 °C at 10 °C/min and maintained for 5 min. The standard compound was dissolved in 56% (v/v) ethanol solution prepared by ethanol and ultra-pure water to obtain the mixed standard stock solution. It was then diluted into a series of different concentration gradients. After mixing the standard solution (1.0 mL) with the internal standard solution, the mixed solution was analyzed by GC-FID.

2.6. Odor activity values (OAVs) calculation

OAVs were used to determine whether a compound has an aromatic contribution. OAV was calculated by dividing the compound's concentration (C) by the odor threshold (OT). Compounds with an OAV ≥ 1 are considered to have an aroma that contributes to their aroma characteristics.

2.7. Aroma recombination

The 56% (v/v) ethanol solution was used as the base solution. The 37 key compounds selected from OVA > 1 compounds in the typical base liquor sample. Then, these key compounds were added to the base solution according to the original concentration, and the recombinant sample was obtained after mixing. The recombinant samples and their corresponding typical base liquor samples were evaluated by descriptive sensory analysis.

2.8. Statistical analysis

The statistical analyses were carried out with one-way analysis of variance using SPSS (SPSS, Inc., USA), and comparisons were considered statistically significant if the p value was lower than 0.05. OPLS-DA was carried out with SIMCA version 14.0. was applied to visualize experimental data between compounds and aromas identified by Pearson correlation analysis, with $r > 0.6$ and significance $p < 0.05$. TBtools visualized the compound amounts and OAV values of *Jiangxiang*, *Chuntian*, and *Jiaodixiang* typical base liquors (Chen et al., 2020).

3. Results and discussion

3.1. Sensory analysis of the Jiangxiang, Chuntian, and Jiaodixiang typical base liquors

The sensory analysis showed significant differences in the flavor profiles of *Jiangxiang*, *Chuntian*, and *Jiaodixiang* typical base liquors from five production regions. (Fig. 2). The *Jiangxiang* typical base liquor has high aroma expressions in *jiang* (SV: 3.86 ± 0.72) and caramel (SV: 2.83 ± 0.29) (Fig. 2A). The *Chuntian* typical base liquor has high aroma expressions in ethanol (SV: 3.41 ± 0.16), sweet (SV: 3.06 ± 0.29), and fruity (SV: 2.43 ± 0.16) (Fig. 2B). The *Jiaodixiang* typical base liquor has high aroma expressions in pit mud (SV: 3.57 ± 0.7) (Fig. 2C).

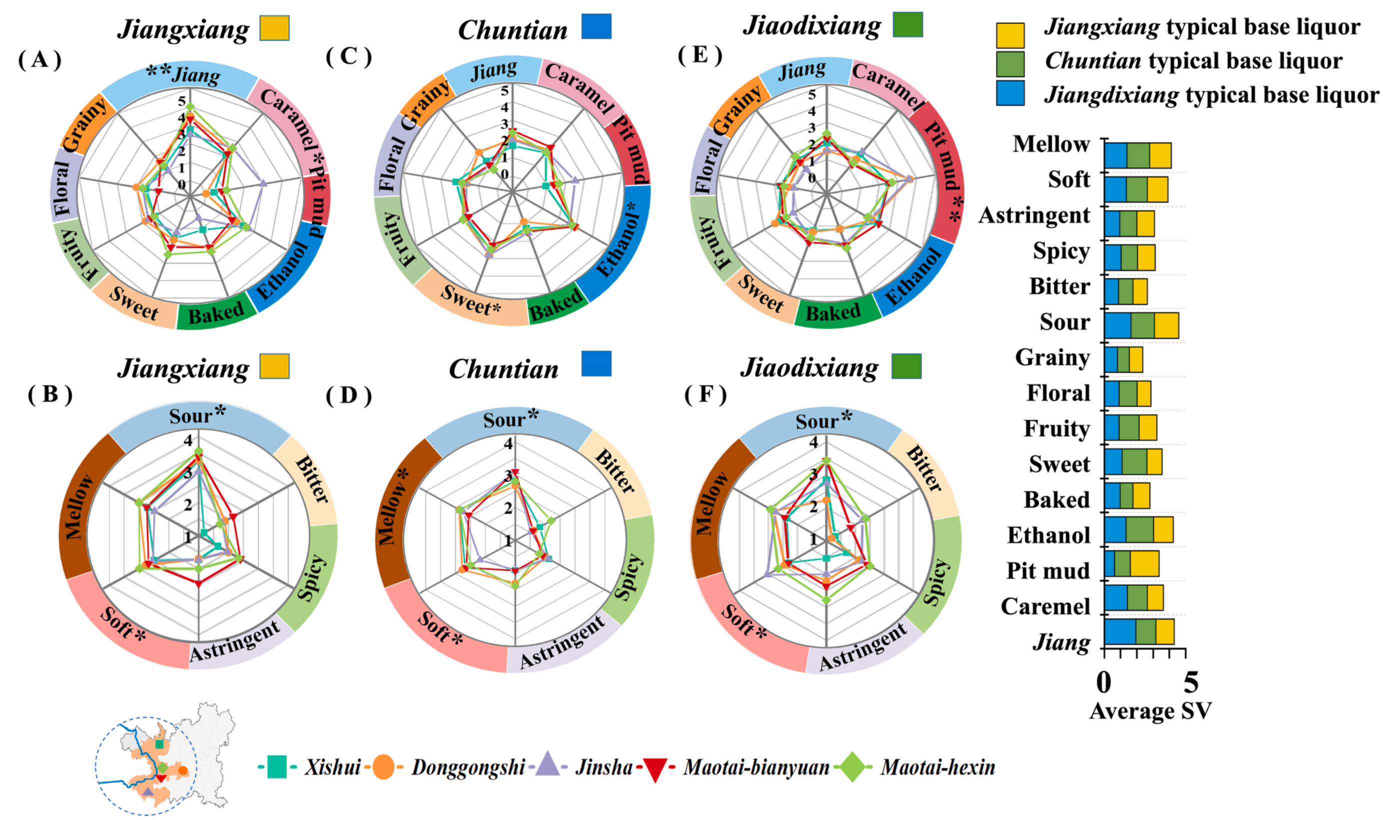

Fig. 2. Sensory analysis of the three typical base liquors in different regions. (A, C, and E. Aroma score plots of three typical base liquors in different regions. B, D, and F. Taste score plots of three typical base liquors in different regions. G. Indicates the average sensory scores of different typical base liquors in different regions. The radar chart in the middle indicates the aroma score or taste score of different regions in the typical base liquor, and the garden in the outer circle indicates the average aroma or taste score of different regions in the typical base liquor. "*"and "**" in Fig. 2 represent $p < 0.05$, and $p < 0.01$, respectively).

In addition, the aroma characteristics of the three typical base liquors exhibit regional variation. The aroma characteristics of the typical base liquor did not differ much in terms of *jiang* and ethanol, but the aroma characteristics of pit mud varied in different regions (Fig. 2). The aroma of pit mud varies among regions, likely due to the variations in the content of the important compounds (Bayram & Kayalar, 2018). In contrast, the taste of the typical base liquor shows less discrepancy in different regions than the aroma. It may be that nonvolatile compounds with taste activity vary little (Jia & Ma, 2023). Among them, sour (SV: 3.29 ± 0.23) and mellow aromas (SV: 2.79 ± 0.22) are prominent in the *Jiangxiang* typical base liquor, the *Chuntian* typical base liquor exhibits soft (SV: 2.56 ± 0.23) and astringent aromas (SV: 2.08 ± 0.24), and sour (SV: 2.95 ± 0.51) and mellow tastes (SV: 2.66 ± 0.24) are prominent in the *Jiaodixiang* typical base liquor. This shows that compounds with major aroma contributions were not consistent in their intensity of contribution across typical base liquor (Fig. 2).

The flavor profile was unique in the typical base liquor, which was related to the differences in the types of flavor compounds produced by microorganisms in the different fermentation locations. The high temperature in the upper layer of the pit promotes heat-resistant *Bacillus* metabolism, resulting in the production of a large number of metabolites that contribute to the *jiang* aroma. Additionally, the content may cause differences in the aroma contribution of different flavor compounds in the three typical base liquors body. The bitter taste was mainly due to the high production of alcohol compounds (Luo et al., 2020). In different regions, MH and MB in the midstream region have a very prominent *jiang* aroma and sour taste in the *Jiaingxiang* typical base liquor, XS in the downstream region has a prominent floral aroma in the *Chuntian* typical base liquor, and JS and DGS in the upstream region have a very prominent pit mud aroma in the *Jiaodixiang* typical base liquor (Fig. 2C).

3.2. Volatile compounds profiled in the three typical base liquors

A total of 201 kinds of major volatile compounds were identified, including esters (89 kinds), alcohols (23 kinds), acids (19 kinds), aromatics (28 kinds), alkanes (9 kinds), pyrazines (2 kinds), ketones (9 kinds), furans (5 kinds), aldehydes and acetals (6 kinds), phenols (3 kinds), sulfur compounds (2 kinds), and other compounds (13 kinds) (Fig. 3). The total content of compounds and the number of substances differed among the three typical base liquors from each region. In terms of type and total content, most flavor compounds are found in the *Jiaodixiang* typical base liquor (11873.26 ± 0.13 mg/L), while flavor compounds are low in the *Jiangxiang* (11370.32 ± 0.21 mg/L) and the *Chuntian* typical base liquors (10649.84 ± 0.16 mg/L).

Different categories of compounds are present in different contents in the typical base liquor. More acid compounds (441.72 ± 0.17 mg/L) were found in the *Jiangxiang* typical base liquor in each region than in the other typical base liquor (Fig. 3c). MH (446.39 ± 0.11 mg/L) and MB (549.89 ± 0.02 mg/L) had more types and contents of acids. Five high-content compounds included acetic acid, butyric acid, and caproic acid, which are the primary acids in sesame-flavor *Baijiu*, which contains acetic acid that produces ethyl acetate with alcohol compounds and demonstrates the caramel aroma in *Baijiu* (Li et al., 2019). Acid production has been related to pyruvate metabolism during the yeast fermentation of *Baijiu* (Wang et al., 2022). Yeast produces large amounts of ethanol, which is converted to lactic acid by *Lactobacillus*, and *Lactobacillus* serves as the core functional microorganism. As facultative anaerobes, *Lactobacillus* was more abundant in the upper part of the pit, where it could have more contact with oxygen. Thus, acid compounds (441.72 ± 0.17 mg/L) produced in the *Jiangxiang* typical base liquor were dominantly produced by *Lactobacillus*.

Moreover, except for *Lactobacillus*, the bacteria from the high-

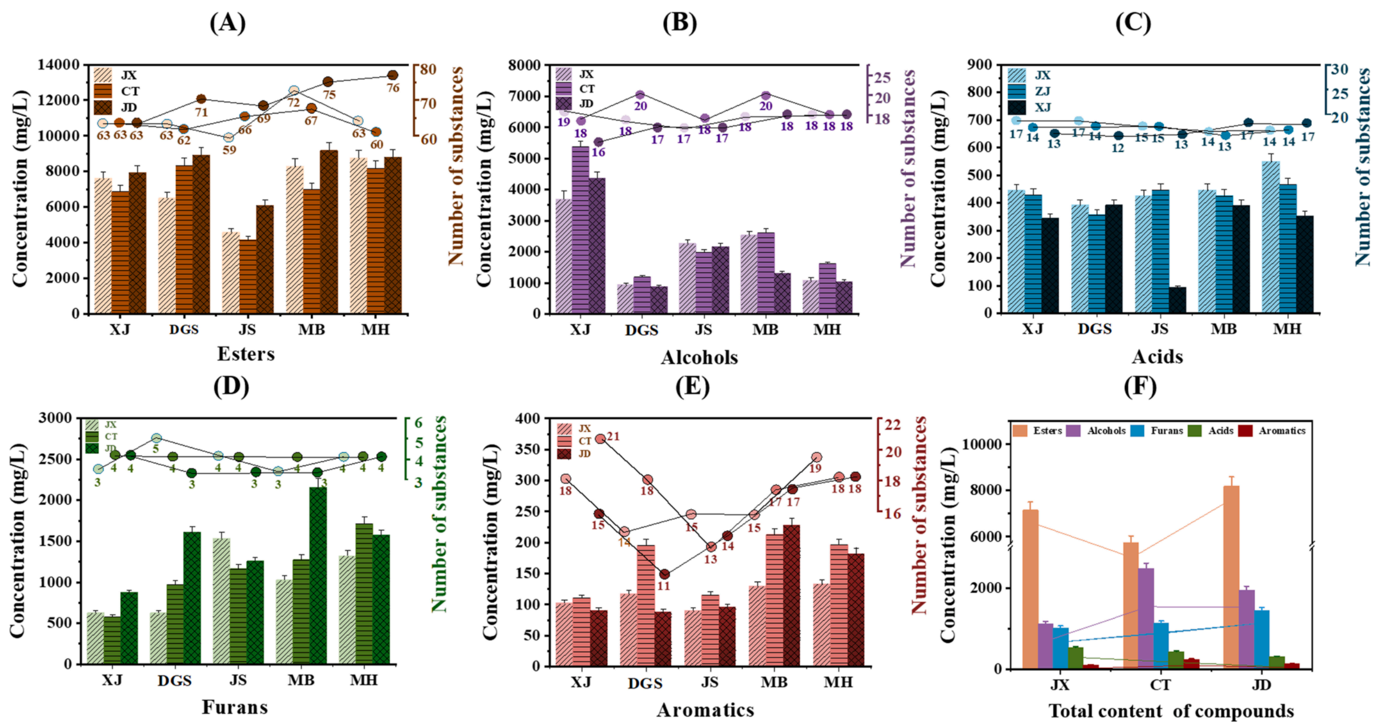

Fig. 3. The content and number of high-content compounds in different regions of the typical base liquor (A–E); The average content of high-content compounds in the typical base liquor (F). (The bar graph represents the total content of each type of compound and the line graph represents the type of compound).

temperature *Daqu* also produce acids when added to the fermented grain. For instance, *Acetobacter* will oxidizes glucose to produce acetic acid and a small amount of alcohol in pit fermentation (Zhang, Hong, Yu, Wang, Li, & Liu, 2023). The amount of *Lactobacillus* in the upper layer of the pit is greater than that in the middle and fewer of the pit, which explains the high contents of acid (441.72 ± 0.17 mg/L) in the *Jiangxiang* typical base liquor (Song, Du, Zhang, & Xu, 2017).

Except for the JS regions, the *Chuntian* typical base liquor from each region contained more alcohol (1989.71 ± 0.55 mg/L) than the other two typical base liquors (Fig. 3b), with the typical base liquor from XS containing the most alcohol (1989.71 ± 0.55 mg/L). The alcohol compound (mg/L) in the typical base liquor may be related to the dominant pit fermentation strains, such as *Zygosaccharomyces bailii*, which are high producers of alcohol and flavor substances. The high alcohol compounds (1989.71 ± 0.55 mg/L) were produced by the fermentation intermediate product α-keto acid, which can be decarboxylated and dehydrogenated if the yeast lacks a suitable nitrogen supply or accumulates an excessive number of amino acids (Fang, Du, Jia, & Xu, 2018). However, excess of higher alcohols causes the original aroma compounds to give off a bad odor, such as *n*-propanol and 2-methylbutanol, which present a floral aroma (Fig. 3a) and will present a bitter taste after excess (Fang et al., 2018; Niu, Zhang, Xiao, & Zhu, 2020).

The *Jiaodixiang* typical base liquor shows the largest contents and types of volatile compounds. The ester compounds (8181.64 ± 0.15 mg/L) were higher than those in other typical base liquor from each region, especially the region of DGS (Fig. 3a). With less oxygen at the lower layer of the pit, yeast is reduced and carries out anaerobic fermentation, producing alcohol and carbon dioxide by oxidizing glucose under anoxic conditions, which facilitates the formation of esters (Xie, Zhang, Kang, & Yang, 2021). Furthermore, the low temperature in the pit's lower layers limits the hydrolysis reaction of esters, inducing the accumulation of esters, which in turn promotes a large accumulation of esters (Wei, Lu, Nie, Li, Du, & Xu, 2023). The content of furan compounds was high in the *Jiaodixiang* typical base liquor (1255.03 ± 0.47 mg/L), which was related to high furfural contents. The levels of furan compounds in the fermented grains in the lower layer were lower than those at the upper layer. However, there are more furan compounds (1255.03 ± 0.47 mg/L) in the *Jiaodixiang* typical base liquor distilled from fermented grains. The main reason is that poly-pentose in turn forms furfural at high temperatures, and the higher acidity of the fermented grains themselves contains a large amount of poly-pentose, and the poly-pentose is also produced from the cellulose in the rice husk added to the distillation vessel (Bains, Kumar, Chauhan, & Das, 2022).

The differences in the dominant microorganisms at the different locations of the fermented grains in the pit, which result in the three typical base liquors from different regions, differ in the main high-content compound. To a lesser extent, the different fermentation environments (oxygen, acidity, etc.) cause the same microorganisms to carry out diverse metabolisms and produce various compounds (Pan et al., 2023). The distillation process also leads to changes in the compounds. In particular, the compounds with a high content in the fermentation grains may be reduced during the distillation process.

3.3. Analysis of key aroma compounds in three typical base liquors

Not all volatile substances contribute to fragrance. The essential ingredients must be thoroughly identified and how they affect the flavor of the typical base liquor must be considered. The OAVs of the volatile compounds were calculated to further investigate the key aroma compounds in the typical base liquor (Liao, Yan, Wang, Meng, Zhang, & Tong, 2020). The typical base liquor resulted in a total of 37 key aroma compounds with OAV > 1 (Supplementary Table S1), which were divided into three groups (Fig. 4). Among them, key aroma compounds with OVA > 2 include ethyl laurate (Average OAV: 1.64), ethyl nonylate (Average OAV: 3.51), and acetic acid (Average OAV: 1.66), etc. Compounds with OAVs ranging from 3 to 100 were found in isobutanol (Average OAV: 3.84), 2,3,5-trimethylpyrazine (Average OAV: 4.02), and ethyl butanoate (Average OAV: 54.69). Ethyl acetate (Average OAV: 122.68), ethyl hexanoate (OAV: 418.37), and ethyl octanoate (OAV: 774.38), etc. belong to the group with OAV > 100. Each compound contributes to flavor intensity differently. Acetic acid has a sour taste, but ethyl acetate has a fruity and floral aroma (Fan & Qian, 2006).

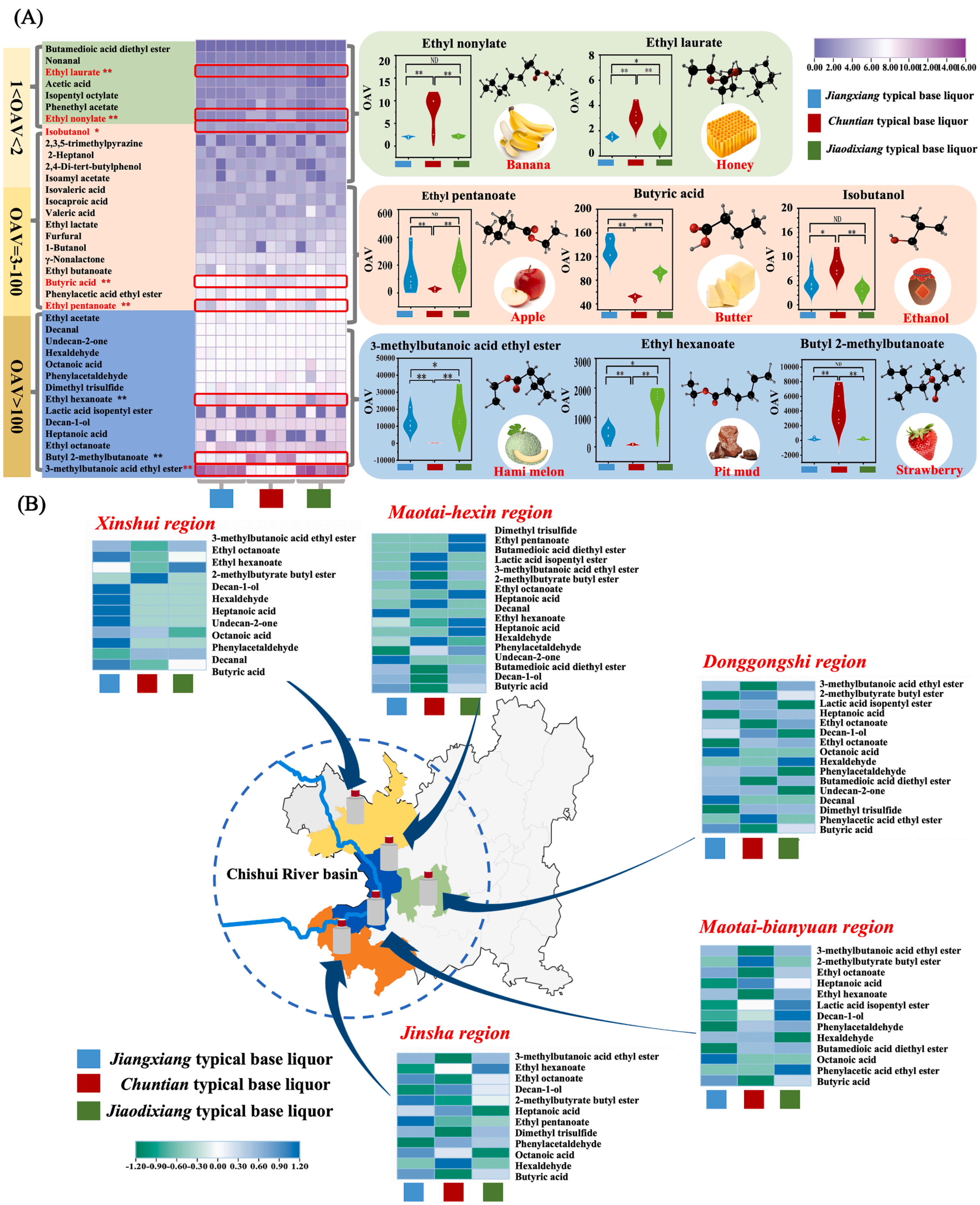

Fig. 4. The OAV of key aroma compounds in different typical base liquors (A); Top 10 aroma compounds of different typical base liquors in each region (B) ("*"and "**" in Fig. 1 represent p < 0.05, and p < 0.01, respectively).

Isocaproic acid presents a sour taste and has more content in the *Jiangxiang* and *Jiaodixiang* typical base liquors.

Moreover, 8 compounds with significantly ($p < 0.05$) different OAVs were found for the first time in the three typical base liquors. Among them, butyric acid mainly shows an extremely significant large OVA in the *Jiangxiang* (OAV: 102.21) ($p < 0.01$) typical base liquor. *Clostridia* is the main genus of butyric acid and ethyl ester-producing bacteria (Chai et al., 2019). The 3-methylbutanoic acid ethyl ester (fruity aroma) was produced by the biosynthesis of the branched chain amino acid, L-leucine, as a precursor and shows the highly significant largest OAV in the *Jiangxiang* (OAV: 12525.24) ($p < 0.01$) and *Jiaodixiang* (OAV: 16964.21) ($p < 0.01$) typical base liquors. In addition, the characteristic key aroma compounds in the *Chuntian* typical liquor body, including butyl 2-methylbutanoate (fruity aroma) (OAV: 6045.59) ($p < 0.01$) and ethyl pentanoate (fruity and ethanol aroma) (OAV: 92.87) ($p < 0.01$), are produced by the esterification reaction between alcohol and the respective acid by lipase action (Corradini, Costa, Bressani, Garcia, Pereira, & Mendes, 2017). The *Chuntian* typical base liquor exhibits a characteristic sweet taste in sensory analysis. The sweet aroma found in the *Chuntian* typical base liquors due to the presence of alcohols such as isobutanol (OAV: 6.7) ($p < 0.05$). Compared to other fermented foods, the sweet aroma in *Baijiu* comes mainly from alcohols, and the sweet aroma increases with the increase in the number of hydroxyl groups (Kim, Park, & Cho, 2022).

The types of key compounds differed from one region to another, but the contents varied. The 3-methylbutanoic acid ethyl ester from the *Jiangxiang* typical base liquor was less abundant (7.51 mg/L) in JS, which may explain the weaker fruit and *jiang* aromas in JS. The *Jiangxiang* typical base liquor and the *Chuntian* typical base liquor had similar levels of key compounds in the MB and MH in the midstream region of the Chishui River basin. The DGS and JS were similar in the *Chuntian* and *Jiaodixiang* typical base liquors (Fig. 4B).

Based on studies, the main source path of the key compound is also revealed, including (1) the raw materials of the brewing process, such as *Daqu* and fermented grain; (2) the metabolism of microorganisms and the production of secondary metabolites (Chai et al., 2019; Xie et al., 2021); (3) compounds directly catalyzed by various biological enzymes or indirectly catalyzed by biosynthesis, such as some alcohol and ester compounds with branched-chain fatty acids as a precondition for synthesizing compounds; and (4) nonenzymatic reactions in the distillation process, *Baijiu* undergoes degradation or interaction under the influence of heat during the distillation process, generating a large number of new flavor compounds. The key aroma compound, which has distinct source paths and thus exhibits OAVs differently during the fermentation of the typical base liquor, is the cause of the flavor variations across the three typical base liquors.

3.5. Correlation analysis of key aroma compounds with aroma and taste in three typical base liquor

OPLS-DA analysis was conducted based on the 37 key aroma compound determinations to further explore the variations in aroma characteristics among the typical base liquors further (Supplementary Table S1). R2X-R2Y < 0.3 and the regression intercept of Q2 < 0.5 indicated that the OPLS-DA model has a high degree of stability and reliability (Szymańska, Saccenti, Smilde, & Westerhuis, 2012).

The aroma and taste characteristics of the typical base liquor had significant differences (Fig. 5). Of them, the *Jiangxiang* typical base liquor primarily exhibited the flavor characteristics of *jiang*, caramel, mellow, and sour flavors. Since the acid compounds (valeric acid and acetic acid) were positively correlated ($p < 0.05$) with the flavor of *jiang* and sour, it seems that the *jiang* and sour flavors were mainly contributed by acids (Fig. 5a). This result is consistent with the study of flavor compounds in sesame-flavor *Baijiu* (Liu et al., 2014). Positive correlations were found between mellow and isoamyl acetate. The aromas in the *Chuntian* typical base liquor were predominantly ethanol, sweet, and floral, and the taste was predominantly soft. Much of what gives the *Chuntian* typical base liquor its characteristic flavor is alcohols and esters, such as 2,4-di-*tert* butylphenol, isobutanol, ethyl laurate, and isoamyl acetate (Fig. 4b). The *Jiaodixiang* typical base liquor primarily exhibited pit mud and baked characteristics, and the aroma of pit mud was significantly ($p < 0.05$) correlated with ethyl hexanoate.

The flavor style is ultimately expressed by the aroma and taste of a single compound and the interaction between different flavor contributions and the flavor correlation of various compounds. Odors are produced because compounds bind to human odor receptors to generate neural signals that are perceived by humans. A single odor receptor can respond to various compounds, and one compound can activate multiple odor receptors to reveal different aromas and tastes (Fukutani et al., 2023), such as ethyl octanoate, which has presents sweet and ethanol aromas. The compounds with high flavor attributes were associated with higher OAV when combined with the above OAV results, such as butyl 2-methylbutanoate, which had a strong correlation with fruit aroma (OAV: 6045.59). However, the presence of aroma compounds in *Baijiu* has an aroma perception effect, and compounds with low OAV may have cooperative effects with other compounds and have a potentiating effect on odor intensity. Lactic acid has a cooperative effect, which can play a role in lowering the olfactory threshold of ethyl lactate and ethyl acetate (Wang et al., 2022). The actual aroma contribution of the key aroma compound should be systematically examined by aroma recombination (Ferreira, de-la-Fuente-Blanco, & Sáenz-Navajas, 2021). In general, the key compound (OAV > 1) not only distinguishes the three typical base liquors, but some of these compounds, including butyric acid, ethyl laurate, and butyl 2-methylbutanoate, are significantly correlated ($p < 0.05$) with the flavor.

3.6. Aroma recombination of 37 key aroma compounds

There was little difference between the main flavor characteristics of the recombinant and the typical base liquor samples (Fig. 6). The recombinant samples and the typical base liquor samples from different regions showed high similarity. The *jiang* aroma (SV: 4.2–4.8) and caramel flavor (SV: 2.1–3.0) were evident in the recombinant *Jiangxiang* typical base liquor sample. The recombinant *Chuntian* typical base liquor had similar mellow (SV: 3.1–4.0) and sweet (SV: 2.9–3.2) aromas, but other aromas in the recombinant sample were slightly different from the

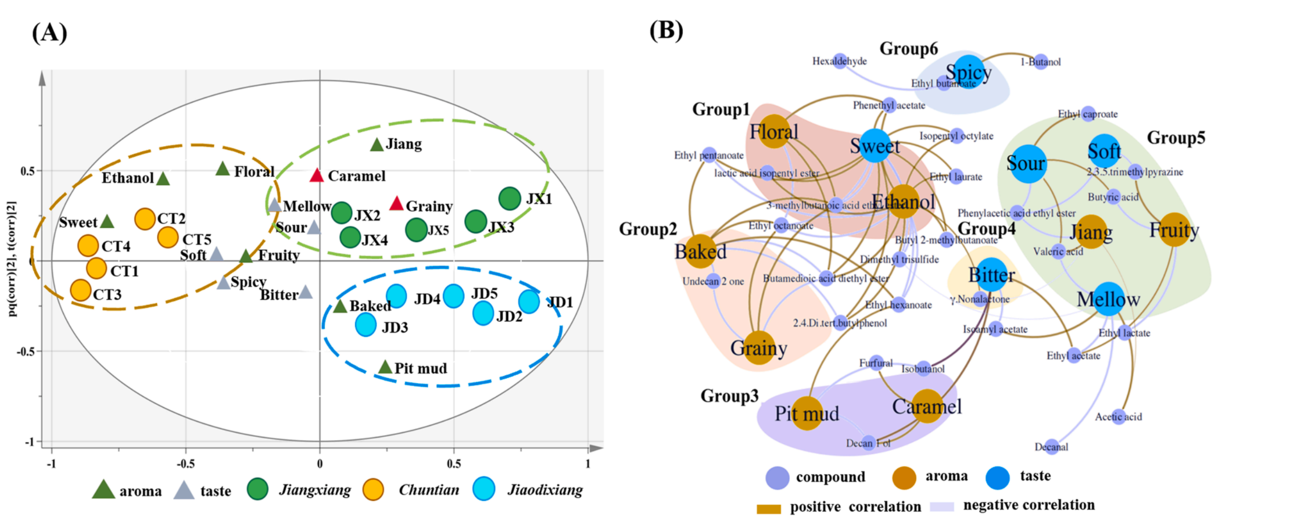

Fig. 5. OPLS-DA analysis of three typical base liquors (A); the network chart of significant ($p < 0.05$) correlations with the aroma and taste in the key compounds (B).

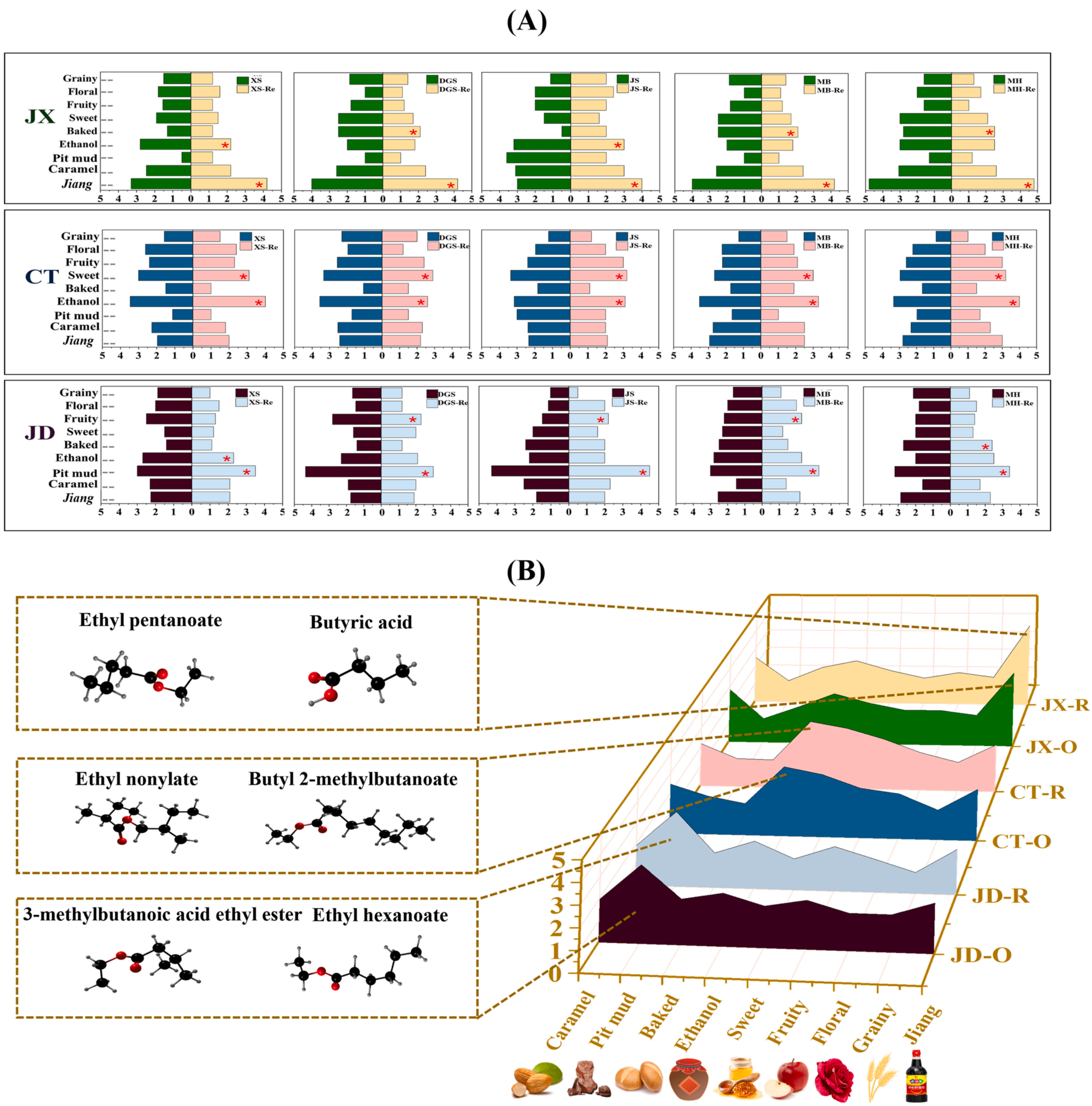

Fig. 6. Comparison of the aroma scores of the original and reconstructed samples (A); the scent profile in recombinant samples and original samples (B). (JX: *Jiangxiang* typical base liquor, CT: *Chuntian* typical base liquor, JD: *Jiaodixiang* typical base liquor; * representing this aroma was significant ($p < 0.05$) in recombinant and original samples).

typical base liquor sample. The difference between the typical base liquor and the recombinant sample might be due to compounds having smaller actual contributions to aroma or the weak synergistic effect of aroma compounds in the recombinant sample (Liu et al., 2021). For instance, ethyl butanoate in the compound and dimethyl trisulfide in the same system exert a masking effect. The pit mud aroma (SV: 3–4.5) and fruity aroma (SV: 1.0–2.8) were well simulated by the *Jiaodixiang* typical base liquor from different regions.

In addition, the recombinant samples from different regions share commonalities. The *Jiangxiang* typical base liquor recombinant sample exhibits the same aroma in MH and MB, while the *Chuntian* typical base liquor recombinant sample has the same aroma in XJ and DGS. The *Jiaodixiang* typical base liquor recombinant sample has the same main aroma in JS and DGS.

The key aroma compounds with OAV > 1 include butyric acid (*jiang*), butyl 2-methylbutanoate (fruity and ethanol), ethyl laurate (floral and sweet), ethyl hexanoate (pit mud), isobutanol (ethanol and sweet), and 3-methybutanoic acid ethyl ester (*jiang* and fruity). They vary significantly ($p < 0.05$) in different typical base liquors and contribute significantly ($p < 0.05$) to flavor t through the aroma recombination.

4. Conclusions

This study revealed the flavor characteristics and key compounds of three typical base liquors (*Jiangxiang*, *Chuntian*, and *Jiaodixiang*) of *Jiang*-flavor *Baijiu*. The 201 compounds in the three typical base liquors were identified, containing primary components such as 89 esters, 23 alcohols, 19 acids, and 28 aromatics. High concentrations of acid, alcohol, and ester compounds were respectively detected in *Jiangxiang* (mainly focused in the MB and MH regions), *Chuntian* (mainly focused in XS region), and *Jiaodixiang* (mainly focused in DGS and JS regions) base liquor. The 37 key compounds were utilized to distinguish three typical base liquors, in which 8 key aroma compounds showed extremely differences in flavor contribution. Butyric acid (OAV: 102.21), butyl 2-methylbutyrate (OAV: 6045.59), ethyl caproate (OAV: 418.37), and ethyl 3-methylbutyrate (OAV: 12525.24) were related to the *jiang*, sweet, and pit mud aromas, which were respective feature of the *Jiangxiang*, *Chuntian*, and *Jiaodixiang* typical base liquors. This study will provide a scientific basis for the investigation of the flavor structure and characterization of *Jiang*-and/or other-flavor *Baijiu*. For future investigations, the synergistic flavor mechanism of key compounds in the typical base liquor should be the primary focus.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 32260560, 32360571); Guizhou Provincial Science and Technology Project (Grant No. ZK [2022] 047); Guizhou Provincial Science and Technology Support Project (Grant No. [2023] 343); Guizhou Provincial Department of Information Industry Project (Grant No. [2020]198); Guizhou University Natural Science Special Scientific Research Fund Project (X2021327); Central Guidance on Local Science and Technology Development Fund of Guizhou Province ([2019]4019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100932.

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